TITLE

TGR5 Agonists of Natural and Synthetic Origin: Advances in Pharmacological Research

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ABSTRACT

Takeda G protein-coupled receptor 5 (TGR5/GPBAR1) is a G protein-coupled receptor activated by bile acids that plays important roles in metabolic control and immune modulation. TGR5 is expressed broadly in the liver, intestine, adipose tissue, and nervous system and controls glucose and lipid metabolism, energy expenditure, bile acid homeostasis, and inflammation. Stimulation of TGR5 inhibits NF-κB signaling, regulates the NLRP3 inflammasome, and stimulates glucagon-like peptide-1 secretion, which makes it a candidate of therapeutic benefit for obesity, type 2 diabetes, non-alcoholic fatty liver disease, and inflammatory diseases. Natural and synthetic agonists, including bile acid derivatives, INT-777, and INT-767, have demonstrated effectiveness in preclinical models. This review emphasizes the organization, distribution, signaling pathways, and pharmacology of TGR5, with a focus on its potential as a drug target. More work must be done to maximize agonist selectivity and safety to open the door for new therapies at the interface of metabolism and inflammation.

KEYWORDS

Bile acid receptor; Glucose and lipid metabolism; Inflammation; Metabolic regulation; TGR5 (GPBAR1); TGR5 agonists

INTRODUCTION

Takeda G protein-coupled receptor 5 (TGR5), also known as Gpbar1, membrane-type bile acid receptor (M-BAR), or GPR131 is a G protein-coupled receptor that is best known for its activation by bile acids.[1]

Since its identification in 2002, TGR5 has been found to be ubiquitously expressed in humans and animals, and to activate various intracellular signalling pathways upon interaction with bile acids.[2]

In animals, initial experimental studies revealed that TRG5 activation plays a role in the regulation of basal metabolism and energy expenditure; TGR5 is now recognized as a potential target for the treatment of metabolic disorders, such as type 2 diabetes. In addition,

the ability of TGR5 to lower the levels of proinflammatory cytokines in monocytes has opened new insights into the modulatory role of bile acids in pathology where inflammatory processes play a central role, including colitis and atheroma development. TGR5 activation has also been shown to influence experimental cholesterol gallstone formation in mice and found to stimulate bicarbonate secretion by cholangiocytes.[2]

TGR5 has been found to regulate a number of specific processes, including energy expenditure and glucagon-like peptide-1 release. Other actions in which TGR5 is implied range from regulating bile acid homeostasis to playing a role in the nervous system.[1]

1.STRUCTURE, DISTRIBUTION, LOCATION AND METABOLISM OF TGR5:

1.1 DISCOVERY:

In 1999, the field of bile acids experienced a change in thinking when bile acid function moved beyond that of digestive surfactants. Bile acids were found to be signalling molecules, acting as hormones and regulatory ligands. This paradigm shift was spurred by the discovery of the dominant nuclear receptor for bile acids, the farnesoid X receptor (FXR). Since then, convergent studies have established that FXR is involved in the regulation of genes acting in multiple metabolic pathways. Three years after the discovery of FXR,described a cell membrane G-protein coupled receptor (GPCR) that was activated by bile acids. This receptor is generally named the membrane-bile acid receptor (M-BAR) or TGR5, rather than the Gprotein bile acid-activated receptor (GP-BAR1). It was first discovered by Japanese scholar Takaharu Maruyama in 2002. As a membrane receptor, TGR5 is widely distributed in tissues and organs of humans and rodents, including the liver, gallbladder, intestine, kidney, spleen, brain, skeletal muscle, brown adipose tissue (BAT), etc. [3]

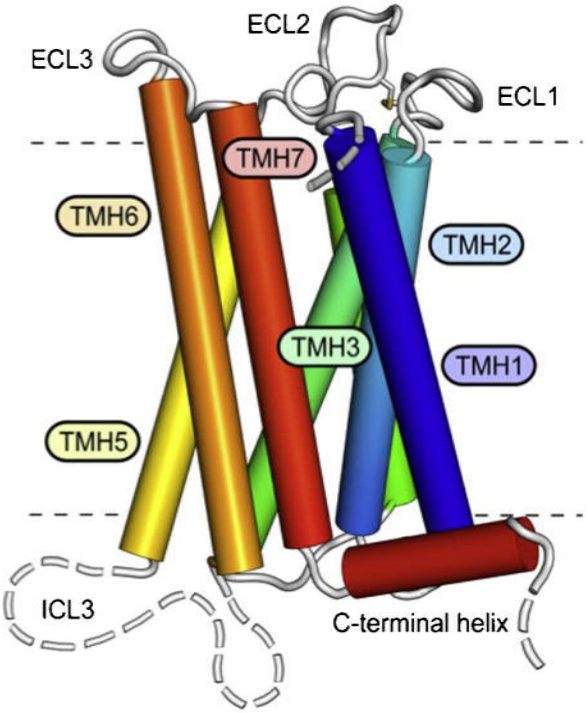
1.2 Nomenclature and Classification:

TGR5 is classified as the founding member of a subclass of GPCRs dedicated to bile acid receptors. The nomenclature "TGR5" reflects its function as a receptor that mediates the effects of bile acids, while "GPBAR1" is another name used interchangeably to denote its role within the broader GPCR family.[4] The TGR5 gene is located on chromosome 2q35 in humans and encodes a protein consisting of 330 amino acids that forms seven transmembrane domains, a characteristic feature of GPCRs.[5]

1.3 LOCATION:

TGR5 receptors are primarily located in gastrointestinal tracts where bile acid functions are most prevalent. They can also be found throughout the body, including the nervous system, immune system, and various muscle groups, aiding in the tasks that are relevant to their respective locations.[6]

1.4 STRUCTURE:



3D structure of TGR5.

The receptor comprises seven transmembrane helices (TMH1-7), three extracellular loops (ECL1-3), contributing to ligand binding, and three intracellular loops (ICL1-3) involved in mediating the signal to downstream signalling molecules. ICL3 and the N- and C-terminal segments are structurally flexible and disordered.[2]

1.5 Synthesis and Metabolism of bile acid:

Pathways of bile acid synthesis:

Cholesterol degradation to bile acids in the liver can be initiated by either cholesterol 7α-hydroxylase (CYP7A1) of the classic (neutral) pathway, or by mitochondrial sterol 27-hydroxylase (CYP27A1) of the alternative (or acidic) pathway. In the classic pathway,

modification of the sterol nucleus including saturation of the double bond, epimerization of the 3β-hydroxyl group, and hydroxylation at the 7α and 12α-positions precedes oxidative cleavage of the side chain. In the alternative pathway, side-chain oxidation precedes steroid ring modification.[7]

1.51 The classic bile acid biosynthetic (neutral) pathway

The classic bile acid synthesis pathway consists of a cascade of fourteen steps catalyzed by enzymes located in the cytoplasm, microsomes, mitochondria and peroxisomes. Fig. 1 is an abbreviated version of this complex metabolic pathway. Detailed description of the enzymes involved and reactions catalyzed can be found in a recent review [1]. In the liver, cholesterol is converted to 7α-hydroxylcholesterol by a microsomal enzyme, cholesterol 7α-hydroxylase, the rate-limiting enzyme of the pathway, which is then converted to 7α-hydroxy-4 cholesten-3-one by a microsomal 3β-hydroxy-Δ5-C27-steroid dehydrogenase/isomerase (HSD3B7). Two cytosolic enzymes, Δ4–3-oxosteroid-5β-reductase (AKR1D1) and 3α-hydroxysteroid dehydrogenase (AKR1C4), reduce 7α-hydroxy-4-cholesten-3-one to 5β-cholestan-3α,7α-diol, a precursor of chenodeoxycholic acid (CDCA). For the synthesis of cholic acid (CA), 7α-hydroxy-4-cholesten-3-one is first hydroxylated at the C-12 position by a microsomal sterol 12α-hydroxylase (CYP8B1), and then reduced to 5β-cholestan-3α,7α,12α-triol by AKR1D1 and AKR1C4. Mitochondrial sterol 27-hydroxylase then oxidizes the steroid side-chain of 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol (Fig. 1). This enzyme incorporates a hydroxyl group to the C27 position, which is subsequently oxidized to an aldehyde and then to a carboxyl group. The products, 3α,7α,12α-trihydroxy-5β-cholestanoic acid and 3α,7α-dihydroxy-5β-cholestanoic acid, respectively, are ligated to coenzyme A by bile acid CoA ligase activity catalyzed by either a bile acid CoA synthetase (BACS) or very long chain acyl CoA synthetase homology 2. The cholestanoyl-CoAs are subsequently transported into peroxisomes where the side-chain is shortened by one cycle of β-oxidation to release a propionyl-CoA, and the product cholyl-CoA or chenodeoxycholyl-CoA. Four peroxisomal very long chain fatty acid β-oxidation enzymes, 2-methylacyl-CoA racemase, branched-chain acyl CoA oxidase 2, D-type bifunctional enzyme, and thiolase 2 (also known as sterol carrier protein κ) are involved in β-oxidation reactions. To increase the solubility for secretion into the bile, CoA derivatives are conjugated at C24 with either glycine or taurine by bile acid CoA: amino acid N-acyltransferase (BAT). Under physiological pH, bile acids form Na2+ salts, and are referred to as bile salts.[7]

1.52 Alternative bile acid biosynthetic (acidic or sterol 27-hydroxylas) pathway

Cholesterol is also oxidized by sterol 27-hydroxylase (CYP27A1) to 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid. These two compounds are converted to 7α,27-dihydroxycholesterol and 3β,7α-dihydroxy-5-cholestenoic acid, respectively, by oxysterol 7α-hydroxylase (CYP7B1). These oxidized metabolites are produced mainly in the peripheral tissues. Other enzymes involved in the alternative pathway are not well defined although many predicted intermediates of the alternative pathways have been identified in HepG2 cells and human hepatocytes. Since both CYP27A1 and CYP7B1 are expressed in various tissues and only the liver has the complete set of bile acid biosynthetic enzymes, these oxidized sterols must be transported to the liver in order to be converted to bile acids.[7] The relative contribution of the classic and alternative pathways to overall bile acid synthesis is not clear. The classic pathway may be the main pathway that is highly regulated under physiological conditions, whereas the alternative pathway may contribute very little to overall bile acid synthesis under normal condition in humans, but may become the major bile acid biosynthetic pathway in patients with liver diseases. In humans, CA and CDCA are synthesized in about equal amounts. In the mouse and bear, CDCA is converted to muricholic acids (3α,6α/β,7β) and ursodeoxycholic acid (3α,7β) (UDCA), respectively. Muricholic acids and UDCA are soluble and non-cytotoxic. Human livers synthesize very small amount of UDCA. UDCA is a therapeutic drug approved for treating gallstone disease and primary biliary cirrhosis.[7]

METABOLSIM

1.53 Enterohepatic circulation of bile acid

In the human body, the conversion of cholesterol is involved in the homeostasis of cholesterol and prevents the accumulation of cholesterol and triglycerides as well as damage to the liver and other organs. The resulting BAs play a key role in promoting the absorption and distribution of nutrients, regulating metabolism and maintaining balance in the body's enterohepatic circulation. [8]

After cholesterol is successfully converted to BAs, BAs are transported from the tubule membrane to the gallbladder for storage through the bile salt export pump (BSEP). Under the stimulus of feeding, BAs are secreted through the duodenal papilla and act as detergents and signalling molecules in the intestine. Most of the BAs in the intestinal cavity can be

reabsorbed by the apical sodium-dependent bile acid transporter (ASBT) at the end of the ileum. The reabsorbed BAs can be delivered to the portal vein through the organic solute transporter α/β (OSTα/β) in the basolateral membrane of intestinal cells. Then, BAs return to the liver through the portal vein circulation and enter hepatocytes under the action of sinusoidal sodium-taurocholate cotransporting polypeptide (NTCP).[9]

2. TISSUE DISTRIBUTION OF TGR5:

TGR5 is widely distributed in various tissues, including the brain, lung, heart, liver, spleen, pancreas, kidney, stomach, jejunum, ileum, colon, brown adipose tissue, white adipose tissue, and skeletal muscle in the human bodyindicating its widespread distribution and potential roles in various physiological processes.[10]

LIVER: In the liver, TGR5 appears in sinusoidal endothelial cells, bile duct epithelial cells and Kupffer cells.[11]

ADIPOSE TISSUE:TGR5 is mainly expressed in gut epithelial cells and organs centrally involved in metabolism, such as the liver and the adipose tissue (AT).[12]

BRAIN AND INTESTINE: TGR5 is expressed in several tissue types, including the liver, neurons of the enteric and central nervous systems, and is involved in various biological processes such as energy balance, insulin and glucose homeostasis, and intestinal motility.[13]

The neurons of the enteric and central nervous system also express TGR5 and it is involved in the intestinal motility and detection of endogenous neurosteroids in response to bile acids.[13]

3. SIGNALING MECHANISM OF TGR5:

A growing body of literature suggests that TGR5 signaling plays an essential role in hepatic metabolic regulation. Our group recently identified a previously unknown role for hepatocyte TGR5 signaling in regulating whole body glucose homeostasis and insulin sensitivity; however, the underlying signaling pathway through which this occurs remains unresolved. Moreover, other studies have characterized anti-inflammatory, choleretic, proliferative, and protective effects associated with TGR5 signaling in the nonparenchymal cell types of the

liver. For instance, in Kupffer cells and resident macrophages, TGR5 activation dampens the hepatic inflammatory response through the attenuation of LPS-induced cytokine production, via the classical TGR5-cAMP-dependent pathway and also through a reduction in NF-κB-dependent inflammatory responses [15] (Figure 1). TGR5 signaling antagonizes NF-κB by decreasing the phosphorylation of IκBα, the nuclear translocation of p65, and NF-κB DNA binding activity. Through these separate pathways, TGR5 signaling functions to diminish hepatic inflammation.[14]

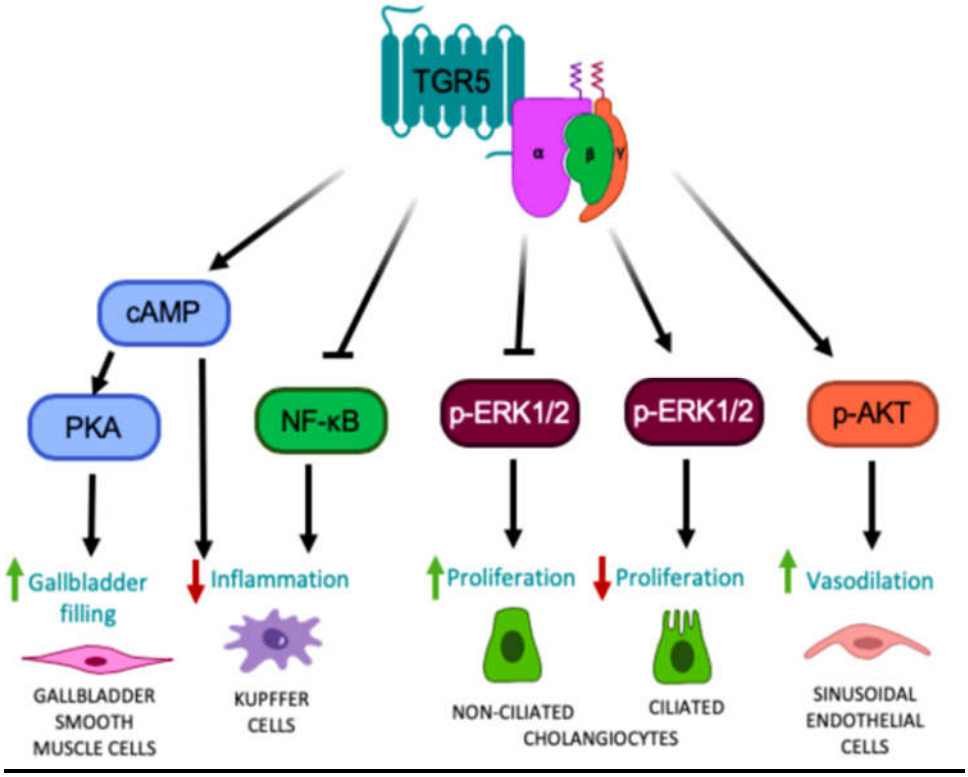


Figure 1.

TGR5-mediated cell signaling pathways in different liver cell types. TGR5 activation leads to increased intracellular cAMP levels, followed by activation of PKA, ultimately leading to altered gene expression, resulting in relaxation of gallbladder smooth muscle cells and increased gallbladder filling. TGR5 signaling in Kupffer cells decreases LPS-induced cytokine production via a cAMP-dependent pathway and antagonizes NF-κB, both resulting in a decreased hepatic inflammatory response. TGR5 signaling in non-ciliated cholangiocytes inhibits ERK1/2 signaling, resulting in increased proliferation; whereas in ciliated cholangiocytes, TGR5 signaling increases ERK1/2 activity to decrease proliferation. TGR5 signaling in liver sinusoidal endothelial cells enhances AKT phosphorylation and increases vasodilation.

3.1 Hepatic TGR5 Signaling Pathway.

The function of TGR5 signaling within the biliary tree has also been rigorously studied. Ligand binding to TGR5 in cholangiocytes induces CFTR-dependent chloride and bicarbonate secretion into bile, which enhances choleresis and forms a bicarbonate umbrella to protect the liver parenchyma from bile acid toxicity. TGR5 signaling in cholangiocytes has also been shown to inhibit, as well as promote, cell proliferation depending on the subcellular localization of the TGR5 receptor. In non-ciliated cholangiocytes where TGR5 is localized to the apical membrane, TGR5 couples to $G\alpha_s$ to increase intracellular cAMP levels, resulting in increased ERK1/2 signaling and promotion of proliferation (Figure 1). In contrast, in ciliated cholangiocytes where TGR5 is localized to the cilia, TGR5 couples to $G\alpha_i$ to decrease intracellular cAMP levels, resulting in increased ERK1/2 signaling and inhibition of proliferation (Figure 1). TGR5 is also expressed in the smooth muscle cells of the gallbladder. TGR5 signaling in these cells activates the cAMP-PKA pathway and causes hyperpolarizationof smooth muscle cells by opening the K_{ATP} channels, ultimately leading to inhibition of gallbladder contractility and increased gallbladder filling(Figure 1). Through these combined functions, TGR5 signaling protects the liver and biliary tree from the cytotoxic effects of bile acids. This is further underscored by the finding that whole body *Tgr5*$^{-/-}$ mice exhibit more severe liver damage, biliary injury, and impaired cholangiocyte proliferation in response to bile acid feeding, compared to wild-type mice.

Furthermore, in liver sinusoidal endothelial cells, TGR5 signaling increases the generation of vasodilatory molecules, nitrogen oxide and hydrogen sulfide. More specifically, TGR5 activation results in PKA-mediated phosphorylation of endothelial nitric oxide synthase leading to increased production of nitric oxide. Additionally, ligand binding to TGR5 has been shown to activate AKT in these cells, resulting in serine phosphorylation of cystathionine γ-lyase and a subsequent increase in hydrogen sulfide production. TGR5 signaling has also been shown to inhibit expression and secretion of endothelin-1, a potent vasoconstrictor.[15] Through TGR5 signaling, the generation of vasodilatory and inhibition of vasoconstrictor molecules serves to modulate liver microcirculation, mitigate portal hypertension and enable adaptation of hepatic blood flow to nutrient uptake.[15]

3.2 NAFLD TGR5 Signalling Pathway.

TGR5 signaling involves multiple pathways, including the cAMP-PKA pathway, which promotes CREB phosphorylation, enhancing mitochondrial function and thermogenesis. TGR5 activation also stimulates ERK phosphorylation, facilitating mitochondrial fission through DRP1 and MFF. Additionally, TGR5 inhibits the NF-kB pathway, reducing inflammatory cytokine production by increasing intracellular cAMP and calcium levels. This multifaceted signaling contributes to glucose metabolism, lipid regulation, and anti-inflammatory effects, playing a crucial role in the prevention and treatment of NAFLD.[16]

3.3 TGR5 ACTIVATION MEDIATES THE NF-kB SIGNALLING PATHWAY AND THE ASSEMBLY OF NLRP3 INFLAMMASOME

3.31 The anti-inflammatory effect of TGR5 is mediated by inhibiting the NF-kB signalling pathway.

TGR5 binds to the Gs protein and subsequently activates the adenylyl cyclase (AC)/cyclic AMP (cAMP)/protein kinase A (PKA) signalling pathway PKA mediates the phosphorylation of cAMP response element-binding protein (CREB) and protein kinase B (AKT). The phosphorylation of CREB mediates repression of NF-κB-responsive elements by a signal transducer and activator of transcription 1 (STAT1)-dependent mechanism. AKT phosphorylation leads to an increase of liver inhibitory protein (LIP), mediated by mTOR.Increased LIP binding to DNA inhibits the transcription of numerous genes coding for chemokines and blunt NF-κB-dependent gene expression.

Furthermore, TGR5 interacts with Gsk-3β, disrupting the stability of the β-catenin destruction complex. Activated PI3K/AKT following the β-catenin signalling pathway inhibits the TLR4/NF-κB pathway and subsequently attenuates the inflammatory responses. Also, TGR5 activation promotes the β-catenin2 binding to IκBα, a substrate of the IκB kinase (IKK), and suppresses IκBα phosphorylation and p65 translocation subsequently, which inhibits the activation of the NF-κB signalling pathway in inflammatory diseases and cancer. The signal transducer and activator of transcription 3 (STAT3) is also a key player in inflammation and cancer since TGR5 activation promotes STAT3 phosphorylation.[17]

3.32 TGR5 activation affects the assembly of the NLRP3 inflammasome

TGR5 ligand binding leads to cAMP-PKA-dependent phosphorylation of NLRP3 at Ser295 in humans (Ser291 in mice). This phosphorylation promotes NLRP3 ubiquitination thus preventing the assembly of the NLRP3 inflammasome, leading to reduced production of pro-inflammatory mediators.Knockdown of TGR5 increased the accumulation of cleaved caspase-8 and NLRP3, abolishing the anti-inflammatory effects of INT-777 after the middle cerebral artery occlusion.However, CDCA induced NLRP3 inflammasome activation and secretion of pro-inflammatory cytokine-IL-1β in macrophages through the TGR5/EGFR signalling pathway. ERK1/2, AKT and JNK are three major signalling cascade downstream of EGFR that activate NLRP3 inflammasome and initiate liver inflammation during cholestasis caused by bile duct ligation.[17] Considering CDCA is a weak agonist for TGR5 and taurocholic acid (TCA), a physiologic ligand for TGR5, fails to elicit a pro-inflammatory effect, the role of TGR5 in this pathway needs more validation. Since TGR5 deletion does not suppress IL-1β secretion totally, it suggests that BAs activate NLRP3 inflammasome acting as damage-associated molecular patterns (DAMPs) to some extent.[17]

3.4 Neuro-humoral signalling by BAs and TGR5 in the gastrointestinal tract

BAs have many patho-physiological effects in the gastrointestinal tract. They regulate hormone secretion, motility, electrolyte and fluid transport, formation and storage of bile, sensory transduction and inflammation. Some of these actions depend on TGR5 and others occur by unknown mechanisms. TGR5 is present in epithelial cells of the intestine and gallbladder, where the high concentrations of luminal BAs may activate the receptor at apical membranes. However, TGR5 is also expressed at the basolateral membrane of certain epithelial cells, and by neurons, smooth muscle cells and immune cells. Although the permeability of the intestinal epithelium to BAs varies between regions and for particular BAs, the secondary BAs (LCA and DCA) are lipophilic and readily cross membranes, and circulating levels of BAs wax and wane during feeding and fasting. Thus, it is probable that absorbed BAs can regulate multiple cells within the gut wall and beyond. The levels of BAs in the intestinal lumen, circulation and tissues also vary due to disease and therapy, which can lead to TGR5-dependent pathology.[18]

3.5 TGR5 signalling pathway in obesity.

3.51TGR5 promotes thermogenesis through the cAMP–PKA–D2 signalling pathway

When there is a significant imbalance between energy intake and consumption—where intake far exceeds consumption—the excess energy is stored as fat, leading to obesity. In this state,

both the number and size of adipocytes increase, resulting in excessive fat accumulation and an imbalance in adipose tissue function. Adipose tissue is categorized into three types: white adipose tissue (WAT), brown adipose tissue (BAT), and beige adipose tissue. WAT primarily stores excess energy, while BAT generates heat. Beige adipose tissue originates from WAT and primarily serves as an energy store, but under specific conditions, it can also generate heat. Studies suggest that BAT plays a crucial role in body weight regulation, energy balance, and glucose metabolism. Its activation enhances energy expenditure, making it a promising strategy for obesity prevention.[19]

The thermogenic function of BAT depends on the abundance of mitochondria in brown adipocytes and the high expression of mitochondrial inner membrane uncoupling protein 1 (UCP1). During mitochondrial ATP synthesis, UCP1 prevents ATP production, instead releasing energy in the form of heat by oxidizing sugars, fats, and amino acids. Additionally, thyroid hormones significantly influence BAT thermogenesis. As the body's largest endocrine gland, the thyroid secretes tetraiodothyronine (T4) and triiodothyronine (T3). T3, the active form, has a higher affinity for thyroid hormone receptors and has been found to stimulate BAT activation and heat production.

T3 promotes mitochondrial autophagy in BAT, increasing mitochondrial turnover while maintaining mitochondrial integrity during thermogenesis. It also induces the expression of thermogenic genes (UCP1, PRDM16, and PGC-1α), fatty acid oxidation genes (CPT1B and ACSL1), and lipolysis genes (PNPLA2 and LIPE), thereby enhancing metabolism. Since T3 is produced from T4 through deiodination, and its serum levels are lower than T4, increasing T3 levels could be an effective obesity intervention. Studies have shown that bile acids (BAs) promote BAT energy expenditure by activating intracellular thyroid hormone conversion, leading to reduced body weight and improved insulin resistance in mice. The BA receptor TGR5 plays a critical role in this process, as it is highly expressed in BAT and promotes T3 conversion when activated.

cAMP, a secondary messenger, is regulated by the positive feedback of TGR5. Activation of TGR5 by its agonists rapidly increases cAMP levels in a dose-dependent manner. In this process, the excitatory G protein subunit Gα on TGR5 induces adenyl cyclase (AC)

activation, leading to the conversion of ATP into cAMP. The rising cAMP levels, in turn, upregulate cyclic-AMP dependent protein kinase A (PKA), which regulates various cellular metabolic functions. Notably, PKA activation enhances the expression of D2, an enzyme responsible for converting T4 into T3, thereby boosting T3 levels and promoting energy expenditure, fatty acid oxidation, and fat metabolism.

This signalling pathway not only plays a role in BAT thermogenesis but also enhances energy consumption in skeletal muscles, where both TGR5 and D2 are expressed. The full elucidation of the TGR5–cAMP–PKA–D2–T3 pathway highlights TGR5's role in BAT thermogenesis and establishes it as a promising target for obesity treatment.[19]

3.6 TGR5 Activation Ameliorates Mitochondrial Homeostasis via Regulating the PKCδ/Drp1-HK2 Signaling in Diabetic Retinopathy

TGR5 signaling involves the regulation of mitochondrial homeostasis through the Ca2+-PKCδ/Drp1 pathway, which reduces mitochondrial fission. Additionally, TGR5 enhances mitophagy by upregulating the PINK1/Parkin signaling pathway. Drp1 is shown to inhibit mitophagy by promoting the separation of hexokinase (HK) 2 from mitochondria, thereby affecting the HK2-PINK1/Parkin signaling. This intricate signaling mechanism highlights TGR5's role in ameliorating endothelial cell dysfunction in diabetic retinopathy.[20]

3.7 Role of the TGR5/TRPV1 signaling pathway in DPNP(Diabetic Peripheral Neuropathic Pain)

3.71 Overview of the TGR5/TRPV1 signalling pathway.

TRPV1 is a cation channel that is highly expressed in dorsal root ganglion neurons; additionally, it is closely associated with DPNP development. TRPV1 activation can contribute to the development of DPNP; contrastingly, inhibition of TRPV1 channels in the dorsal root ganglion can attenuate DPNP symptoms. TRG5 is closely associated with TRPV1 and may act upstream and downstream of the same signaling pathway and jointly participate in the onset and development of DPNP and other related conditions. TRG5 mRNA is exclusively co-expressed with TRPV1 in dorsal root ganglion innervating the bladder; additionally, TGR5 activates the cAMP-cAMP response element-binding protein (CREB) signaling pathway. Under cAMP stimulation, CREB activates TRPV1 promoter transcriptional activity, which in turn induces abnormal peripheral pain through activation of

the downstream signaling protein kinase C. Activated TGR5 modulates itch and provide analgesia by regulating the expression of cation channels such as TRPV1. Further exploration of this pathway would facilitate the elucidation of the mechanisms underlying DPNP and similar conditions as well as the identification of novel treatment targets.[21]

3.72 Relationship between diabetes and TGR5/TRPV1 signalling pathway.

TGR5 is expressed in various tissues, and its main function is to maintain blood sugar level and increase energy consumption. Activated TGR5 can up-regulate the production and secretion of glucagon-like peptide -1(GLP-1) in intestinal endocrine cells and improve glucose homeostasis. Activation of TGR5 can promote lipolysis and energy consumptionand promote metabolic improvement and advanced weight management. TGR5 shows a potential anti-diabetic effect, and TGR5 agonists become potential candidates for the treatment of type 2 diabetes, obesity and other metabolic diseases.

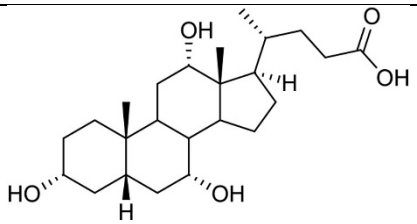
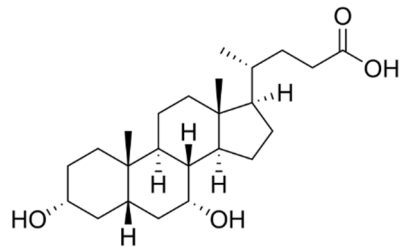
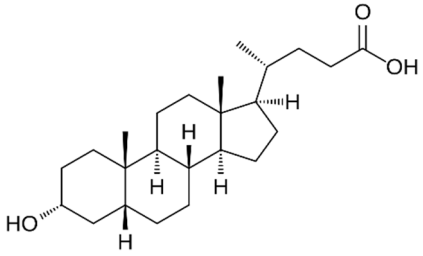
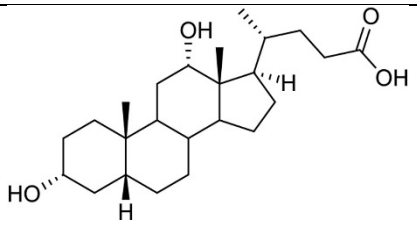
Type 1 diabetes is an autoimmune disease and TRPV1 is potentially associated with autoimmune abnormalities (44). Impaired muscle Ca2+ homeostasis in type 1 diabetic rats was found to be due to TRPV1-mediated attenuation of heat stress tolerance, and capsaicin or other therapeutic strategies that increase Ca2+ accumulation via TRPV1 may be more effective than heat therapy in type 1 diabetic patients. Continuous oral cilostazol treatment was effective in reducing the level of painful peripheral neuropathy in streptozotocin-induced type I diabetic rats, which may be related to denervation of sensory nerves in the epidermis of the hind paw of DM rats, with a significant reduction in TRPV-1-labeled penetrating nerve fibers. Lipid peroxidation products were found to trigger mitochondrial calcium inward flow and mitochondrial dysfunction in endothelial cells in diabetic patients and TRPV1 agonists, and TRPV1 knockout mice were protected from type 1 diabetes-induced endothelial dysfunction and impaired vascular regeneration after arterial injury, and TRPV1 activation may be involved in mediating the process of aberrant lipid metabolism that contributes to the onset of diabetes.TRPV1 channel activation plays a protective role in cardiac oxidative/nitrative stress, mitochondrial function, endothelial function, inflammation, and cardiac energy metabolism in diabetic models, and activation of TRPV1 channels can delay the progression of diabetic complications to a certain extent. In addition, activation of TRPV1 by capsaicin can mediate insulin signaling-independent glucose oxidation and ATP

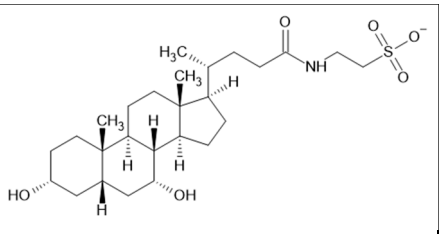
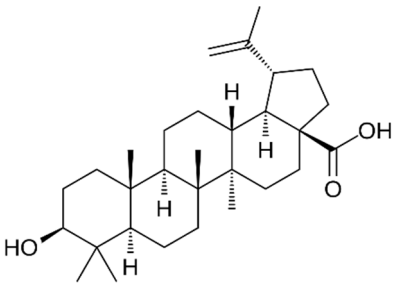
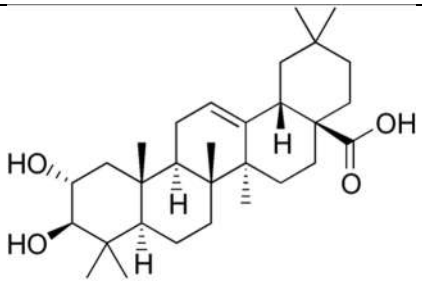
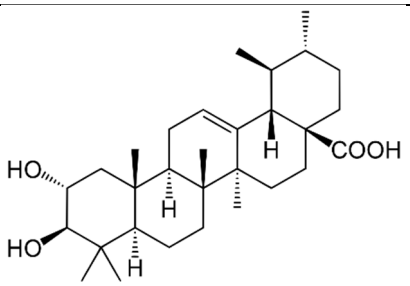
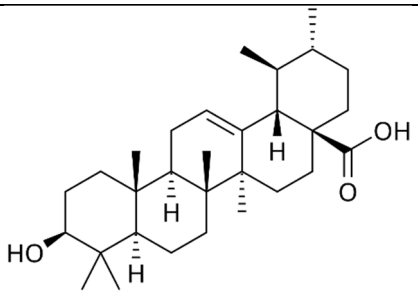
production in mouse skeletal muscle cells, and TRPV1 likewise has potential antidiabetic effects.[21]

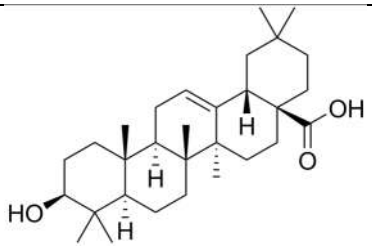
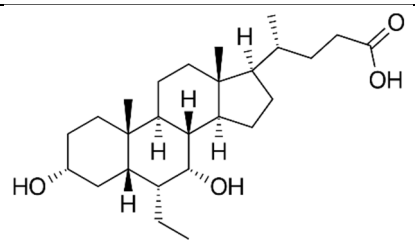
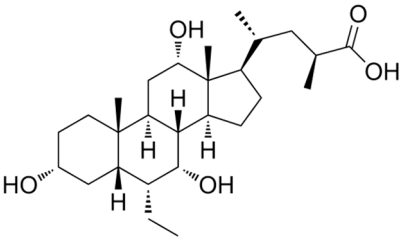
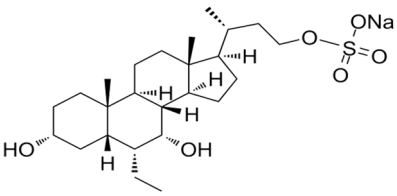
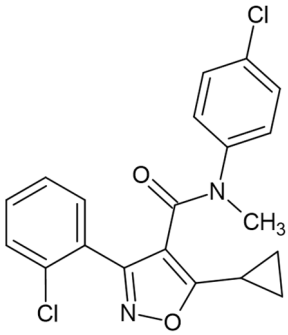
4.PHARMACOLOGY OF TGR5 AGONIST:

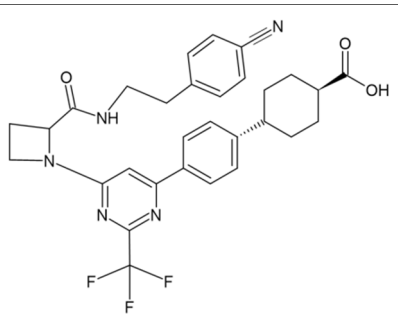
4.1 TYPES OF TGR5 AGONIST

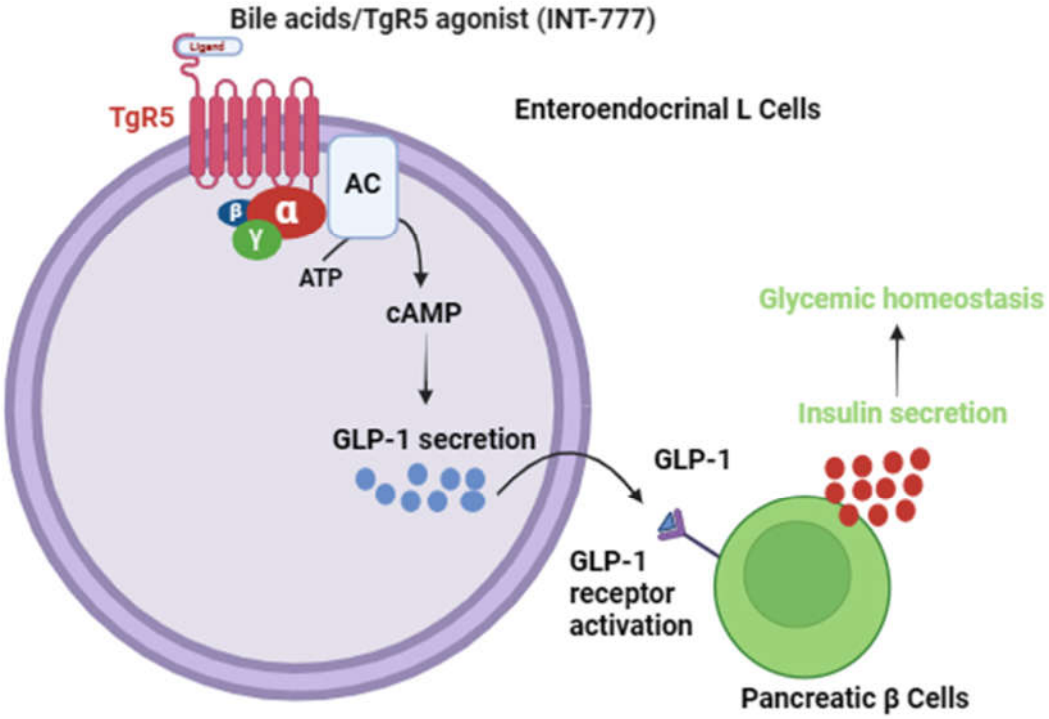
TGR5 receptor gets activated by bile acid-like:

Agonist	Name	Structure
1. Bile Acid		
a. Primary Bile Acid	i) Cholic Acid	
	ii) Chenodeoxycholic Acid	
b. Secondary Bile Acid	i) Lithocholic Acid	
	ii) Deoxycholic Acid	

	iii) Taurochenodexycholic acid	
2. Natural Compound		
	i) Betulinic Acid	
	ii) Maslinic Acid	
	iii) Corosolic Acid	
	iv) Urosolic Acid	

	v) Oleanolic acid	
3. Synthetic Compound		
	i) OCA (obeticholic acid)	
	ii) **INT-777** (6α-ethyl-23(S)-methyl-3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) (CA derivative)	
	iii) INT-767 (6α-ethyl-3α,7α-dihydroxy-5β-cholan-23-sulfate)	
	iv) Isoxazolecarboxamides (3-Aryl-4-isoxazolecarbox	

	v) Trifluoromethyl(pyrimidin-yl)azetidine-2-carboxamide	



General Mechanism of Action of TGR5 Agonist.

GLP-1 glucagon-like peptide-1

CONCLUSION

TGR5 has evolved from being known as a bile acid receptor to being valued as a multifunctional regulator of energy homeostasis, glucose metabolism, immune modulation, and neuro-humoral signaling. Its ubiquitous tissue expression and variant signaling cascades highlight its therapeutic potential in obesity, type 2 diabetes, NAFLD, inflammatory bowel disease, cardiovascular dysfunction, and diabetic complications. The twofold capacity of TGR5 activation to augment metabolic efficacy and inhibit inflammatory responses renders it especially enticing for the treatment of multifaceted metabolic-inflammatory diseases. As

important advances have been made in the discovery of natural and synthetic TGR5 agonists, hurdles persist in attaining selectivity, bioavailability, and reducing off-target effects. Future research ought to concentrate on improving agonist design, delineating tissue-specific signal networks, and establishing prolonged clinical safety. Together, TGR5 constitutes an exciting arena in drug development, providing potential for developing new drugs that engage metabolism and immunity.

ABBREVIATION

Abbreviation	Full Form
TGR5	Takeda G protein-coupled receptor 5
GPBAR1	G protein-coupled bile acid receptor 1
GPCR	G protein-coupled receptor
FXR	Farnesoid X receptor
BAT	Brown adipose tissue
WAT	White adipose tissue
CA	Cholic acid
CDCA	Chenodeoxycholic acid
DCA	Deoxycholic acid
LCA	Lithocholic acid
UDCA	Ursodeoxycholic acid
GLP-1	Glucagon-like peptide-1
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLRP3	NOD-like receptor family, pyrin domain-containing 3
IL-1β	Interleukin-1 beta
PKA	Protein kinase A
AC	Adenylyl cyclase
cAMP	Cyclic adenosine monophosphate
CREB	cAMP response element-binding protein
AKT	Protein kinase B
STAT1	Signal transducer and activator of transcription 1
STAT3	Signal transducer and activator of transcription 3
IKK	IκB kinase
IκBα	Inhibitor of kappa B alpha
PKCδ	Protein kinase C delta
DRP1	Dynamin-related protein 1

Abbreviation	Full Form
HK2	Hexokinase 2
PINK1	PTEN-induced kinase 1
ERK1/2	Extracellular signal-regulated kinases 1 and 2
mTOR	Mechanistic target of rapamycin
EGFR	Epidermal growth factor receptor
JNK	c-Jun N-terminal kinase
TRPV1	Transient receptor potential vanilloid 1
DPNP	Diabetic peripheral neuropathic pain
T3	Triiodothyronine
T4	Thyroxine
D2	Type II iodothyronine deiodinase
CYP7A1	Cholesterol 7 alpha-hydroxylase
CYP27A1	Sterol 27-hydroxylase
CYP7B1	Oxysterol 7 alpha-hydroxylase
CYP8B1	Sterol 12 alpha-hydroxylase
AKR1D1	Aldo-keto reductase family 1 member D1
AKR1C4	Aldo-keto reductase family 1 member C4
BACS	Bile acid CoA synthetase
BAT (enzyme)	Bile acid CoA: amino acid N-acyltransferase
OSTα/β	Organic solute transporter alpha/beta
ASBT	Apical sodium-dependent bile acid transporter
NTCP	Sodium taurocholate cotransporting polypeptide
BSEP	Bile salt export pump
HSD3B7	3β-hydroxy-Δ5-C27-steroid dehydrogenase/isomerase
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
NO	Nitric oxide
H_2S	Hydrogen sulfide
LIP	Liver inhibitory protein
PI3K	Phosphoinositide 3-kinase
DAMPs	Damage-associated molecular patterns
NAFLD	Non-alcoholic fatty liver disease
OCA	Obeticholic acid
INT-777	6α-ethyl-23(S)-methyl-3α,7α,12α-trihydroxy-5β-cholan-24-oic acid
INT-767	6α-ethyl-3α,7α-dihydroxy-5β-cholan-23-sulfate
ER	Endoplasmic reticulum
CFTR	Cystic fibrosis transmembrane conductance regulator
GLUT	Glucose transporter
PPAR	Peroxisome proliferator-activated receptor
UCP1	Uncoupling protein 1

Abbreviation	Full Form
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PRDM16	PR domain containing 16
CPT1B	Carnitine palmitoyltransferase 1B
ACSL1	Acyl-CoA synthetase long-chain family member 1
PNPLA2	Patatin-like phospholipase domain-containing protein 2
LIPE	Hormone-sensitive lipase
GLUT4	Glucose transporter type 4

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